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Metal Chelate Compounds of Glycylglycine and Glycylglycylglycine[1]

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Introduction

Although the interactions of a number of metal ions with glycylglycine and glycylglycylglycine have been studied in aqueous solution (1–4), the only attempts to isolate and identify compounds of this type were reported by Kober and Sugiura (5) for glycylglycine-Cu(II) and by Pfeiffer and Saure (6) for bis(glycylglycino)-Cu(II). There has been considerable variation in the formulation of these metal–peptide chelates, as well as some disagreement in the nature of the functional groups coordinated with the metal. The structure assigned by Kober and Sugiura (5) to the metal chelate formed from copper(II) hydroxide and glycylglycine (formula I) indicates the involvement of the peptide linkage in coordination as well as the amino and acetate groups. Similar involvement of the peptide nitrogen in coordination with a metal ion was suggested by Davies and Waind (1) to account for the fact that the calcium chelate of glycylglycine is much more stable than that of glycine. On the other hand, Klotz *et al.* (2) suggested that coordination of polypeptides with the copper(II) ion takes place only with the terminal carboxyl and amino groups, thus giving rise to long chainlike polymers of the type indicated by formula II. Although Monk (3) did not specify a definite structure for metal chelates of glycylglycine, he implied that the carboxyl group and the α-imido nitrogen atom are involved in coordination with the metal.

The work described in this paper involves the preparation and characterization of a number of metal derivatives of glycylglycine and glycyl-

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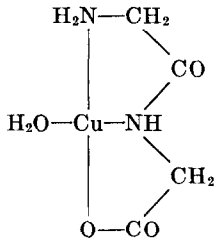

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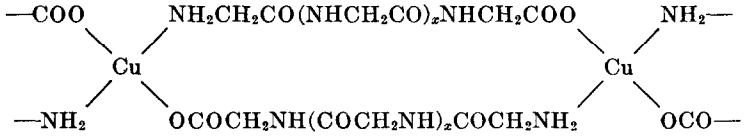


II

glycylglycine, which provide further information concerning the involvement of the peptide linkage in coordination with the metal ion.

Experimental

Peptides

Glycylglycine. Glycylglycine hydrochloride was prepared from glycine anhydride by heating with hydrochloric acid, according to the method of Fischer and Fourneau (7). A solution consisting of 16 g. of the hydrochloride dissolved in 400 ml. of water was passed through a column containing several times the required amount of hydroxy (alkaline) form of Amberlite IR-4B anion-exchange resin. The colorless aqueous eluate was concentrated to one-fifth of its volume under reduced pressure, and the product was crystallized out by the addition of ethanol to the warm solution. A 70% yield of colorless platelets of glycylglycine, which decomposed at 220°, was obtained.

Anal.: Calcd. for $C_4H_8N_2O_3$: N, 21.02; found: N, 20.7.

Glycylglycylglycine. The procedure of Fischer (8) was followed with the following modification. The solution obtained from the ammonolysis of 50 g. of chloroacetylglycylglycine was evaporated under reduced pressure to a small volume (approximately 200 ml.) and was acidified with hydrochloric acid to a pH of about 2. The solution was then passed through a column of the hydroxyl form of Amberlite IR-4B anion-exchange resin, and the column was rinsed with distilled water until the eluate became neutral. The combined effluent solutions and washings were then evaporated to dryness under reduced pressure. The residue was dissolved in 100 ml. of hot water and treated with sufficient ethanol to render it slightly turbid. The colorless microscopic crystals which were obtained when this solution was cooled were recrystallized from aqueous ethanol in a similar manner. The yield was 31 g., 69% of the theoretical amount. The tripeptide decomposed at 242°C. and gave a negative test for chloride ion.

Anal.: Calcd. for $C_6H_{11}N_3O_4$: N, 22.0; found: N, 22.1.

Peptide Chelates

Aquoglycylglycino-Cu(II). Slightly more than 2.4 g. of $Cu(NO_3)_2.3H_2O$ (0.01 mole) was dissolved in water and converted to the hydroxide by treatment with an excess of sodium hydroxide solution. The precipitated hydroxide was washed by decantation until free of inorganic salts, and was then treated in aqueous suspension with 1.32 g. (0.01 mole) of glycylglycine. The excess cupric hydroxide was filtered off, the resulting deep-blue solution was evaporated to a small volume, and the blue copper(II) chelate was crystallized out by the addition of ethanol and ethyl acetate.

Glycylglycylglycino-Cu(II) Hydrate. To an aqueous suspension of slightly more than 0.01 mole of cupric hydroxide, prepared as described above, was added 1.89 g. (0.01 mole) of glycylglycylglycine. The copper chelate was obtained as a blue-purple crystalline solid by the same procedure as that employed for the glycylglycine derivative.

Sodium Bis(glycylglycino) Cobaltate(III) Hydrate. Somewhat more than 2.37 g. (0.01 mole) of $CoCl_2.6H_2O$ was dissolved in water and treated with an excess of sodium hydroxide and hydrogen peroxide. The black cobalt(III) hydroxide thus formed was washed by decantation until the washings were neutral. An aqueous solution of 2.64 g. (0.02 mole) of glycylglycine and 10 ml. of 1.0 *M* sodium hydroxide was then added, whereupon most of the metal hydroxide dissolved and a wine-red solution was formed. After removal of the residual cobaltic hydroxide by filtration, the metal chelate was obtained in crystalline form by evaporation of the solution and the addition of ethanol and dioxane.

Sodium Glycino-glycylglycino-cobaltate(III) Hydrate. To an aqueous solution containing 1.89 g. (0.01 mole) of glycylglycine, 0.75 g. (0.01 mole) of glycine, and 2.37 g. (0.01 mole) of $CoCl_2.6H_2O$, was added 400 ml. (0.04 mole) of 0.1 *M* sodium hydroxide solution. The resulting reaction mixture, which contained a blue precipitate, was cooled to 0° and stirred while 10 ml. of 30% hydrogen peroxide was added. A wine-red solution was formed, with some residual black solid in suspension. The solution was filtered, evaporated to a small volume under reduced pressure, and treated with methanol and acetone, whereupon the metal chelate crystallized out in the form of a finely divided rose-colored solid.

Diammine-glycylglycylglycino-cobalt(III) Hydrate. To a solution of 1.89 g. (0.01 mole) of glycylglycylglycine in 50 ml. water was added 4.02 g. (0.01 mole) of aquopentammine cobaltic bromide. The latter compound only partially dissolved in the reaction mixture. Upon the addition of 30 ml. (0.03 mole) of sodium hydroxide, the suspended material was completely dissolved within a few minutes to form a dark-red solution. The rose-colored cobalt chelate was crystallized from this solution by the addition of methanol and acetone.

Disodium Bis (glycylglycino) nickelate(II) Hydrate. A suspension of somewhat more than 0.01 mole of nickel(II) hydroxide was prepared by treating the equivalent amount of nickel(II) chloride tetrahydrate with excess sodium hydroxide and washing by decantation until the washings were free of inorganic salts. Upon the addition of 2.64 g. (0.02 mole) of glycylglycine and 20 ml. (0.02 mole) of 1 *M* sodium hydroxide, a dark-green solution was formed. After filtration of the slight excess of undissolved nickel hydroxide, a dark-green crystalline chelate compound was obtained upon the addition of methanol and acetone.

TABLE I

Metal and Nitrogen Analyses of Peptide Chelates

Metal	Donors[a]	n_{H_2O}	Analyses[b] *Calculated* Metal %	N %	*Found* Metal %	N %	Ratio N/M
Cu(II)	gg, H_2O	1	29.9	13.2	29.4	13.1	2:1
Cu(II)	ggg	3	20.8	13.8	20.4	13.7	3:1
Co(III)	2gg	3.5	14.6	13.8	14.7	13.8	4:1
Co(III)	g, ggg	6	13.1	12.4	13.1	12.6	4:1
Co(III)	$2NH_3$ggg	3.5	17.1	20.3	16.9	20.4	5:1
Ni(II)	2gg	10	10.8	10.3	11.1	10.3	4:1

[a] gg = glycylglycine; ggg = glycylglycylglycine; g= glycine.

[b] Theoretical calculations are based on composition specified in the first three columns of this table.

Analytical Results

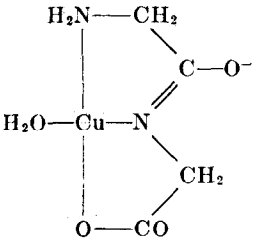
The characterization of the above chelate compounds by analysis of the component elements is given in Table I.

Discussion

For the preparation of the peptides, it was found that passing the hydrochloride through an anion-exchange column was not only more convenient and less expensive than the use of silver oxide suggested by Fischer (7, 8), but the product was more readily obtained in crystalline form as the result of this modification. A number of attempts to produce glycylglycylglycine by Fischer's method resulted in the formation of an oil which failed to crystallize when alcohol was added to the final concentrated solution. Removal of chloride ion by the anion-exchange resin yielded a solution from which the tripeptide rapidly crystallized.

In general the metal chelates of glycylglycine and glycylglycylglycine were most readily prepared by treating the moist metal hydroxide with the appropriate amount of ligand, followed by precipitation with an organic solvent miscible with water. Recrystallization was generally possible by the addition of ethanol or methanol to a moderately concentrated aqueous solution of the compound. In cases where the metal was bound in an anionic complex, sufficient sodium hydroxide was added to form a stable salt. The preparation of diammine-glycylglycylglycino-Co(III) from aquopentammine-Co(III) bromide is believed to be a novel method of synthesis of metal chelates of this type.

The copper(II) complexes prepared from glycylglycine and glycylglycylglycine, containing one mole of ligand per metal ion, are assigned



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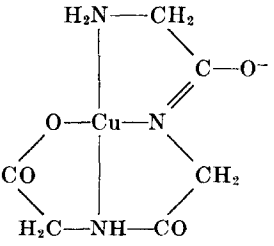

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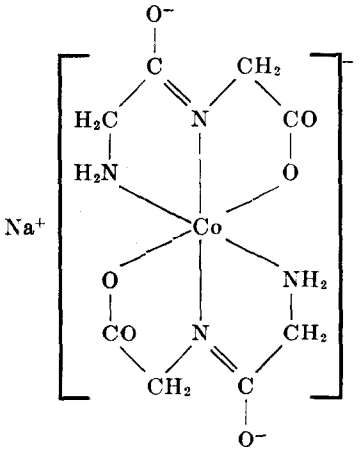

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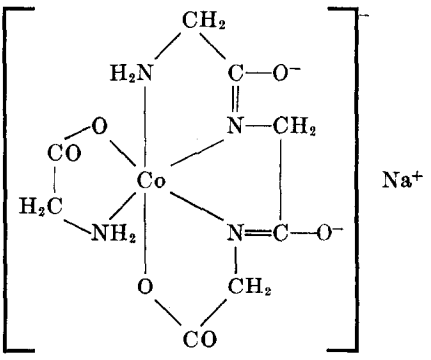

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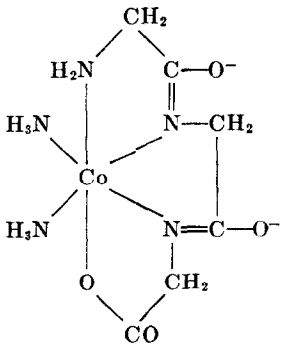

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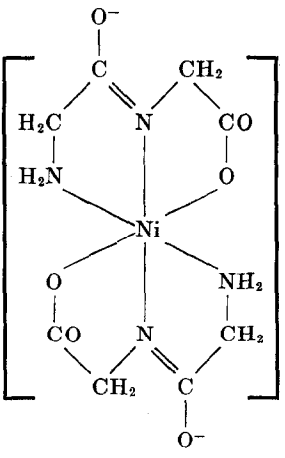


VIII

structures corresponding to formulas III and IV, in which the involvement of the peptide linkages is required to satisfy the coordination requirements of the metal. There are several alternatives to these structures, some of which may be eliminated. In the case of formula III, none of the structures proposed by Davies and Waind (1) or by Klotz (2) can possibly satisfy the stoichiometric requirements established here. The only possible alternative structure is a tautomeric one in which a proton is displaced from the coordinated water molecule rather than from the peptide linkage. Certainly both arrangements of ligands are entirely possible, and the compound may well be a tautomeric equilibrium between these two forms. It should be noted that structure I proposed by Kober and Sugiura (5) can be eliminated in view of our analytical results. On the other hand, this structure probably resembles most closely the arrangement (formula III) which is now believed to be correct.

Formula IV for glycylglycylglycino-Cu(II) is interesting in that while both peptide linkages are involved in coordination of the metal ion, but only one peptide hydrogen ion is displaced. Here again, tautomeric forms, although of a somewhat different nature, probably exist. Because of the

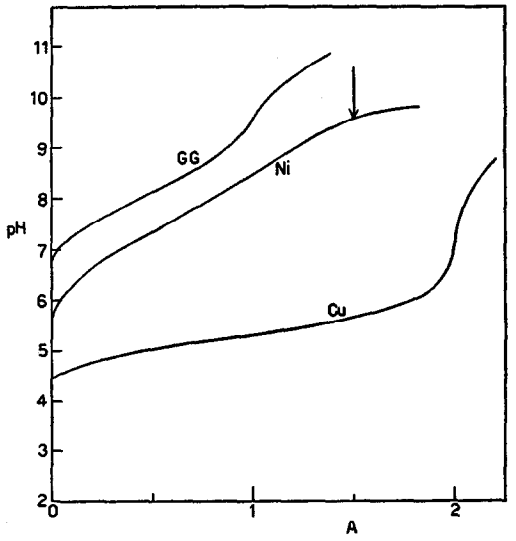


FIG. 1. Titration of 0.001 *M* glycylglycine in the absence of bivalent metals (GG); and in the presence of 0.001 *M* Cu(II) ions, curve Cu; and 0.0005 *M* Ni(II) ion, curve Ni. Arrow indicates precipitation. *A* = moles of potassium hydroxide added per mole of peptide.

presence of water of hydration in the compound isolated, it cannot be definitely stated that water is not involved in coordination with the metal ion. This is believed to be unlikely, however, in view of the fact that the utilization of less than four donor groups of the ligand in metal coordination would probably lead to the requirement of more than one mole of ligand for the solubilization of a mole of copper(II) hydroxide. This would be evidenced by a deviation of the nitrogen to metal ratio from the observed value of 3:1.

The conclusion that the peptide hydrogen is displaced when the nitrogen becomes coordinated with the metal ion is strengthened by the copper titration curves shown in Figs. 1 and 2. In Fig. 1, the neutralization of two protons occurs as a single step when one mole of copper(II) ion combines with one mole of glycylglycine. It would be logical to conclude here that the metal chelate indicated by formula III forms in this step, since the elimination of a hydrogen ion from an aquated water molecule usually occurs as a separate step (i.e., at higher pH).

Perhaps the best proof of the loss of peptide hydrogens is the coordination of the peptide linkage with a metal ion is the 3-proton displacement from glycylglycylglycine seen in Fig. 2. In this case both of the available

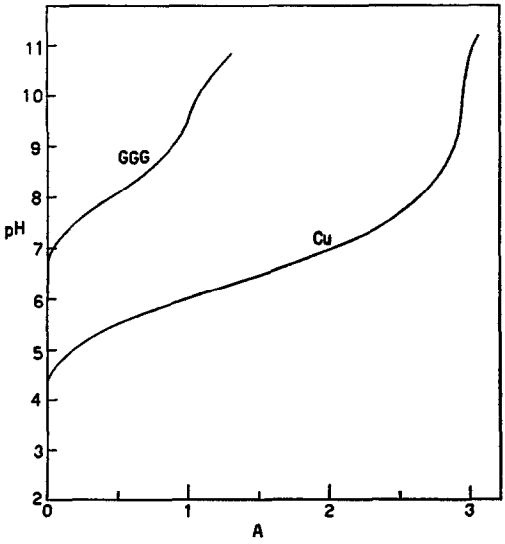


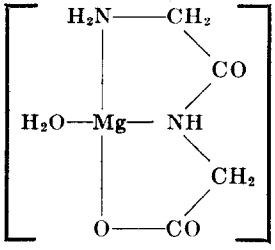
FIG. 2. Titration of 0.001 *M* glycylglycylglycine in the absence of bivalent metals (GGG) and in the presence of 0.001 *M* Cu (II) ion. *A* = moles of potassium hydroxide added per mole of peptide or copper(II) salt.

peptide hydrogens are displaced. It is inconceivable that a relatively rare dihydroxy complex would form in this particular case, while only a monohydroxy complex forms with glycylglycine. This is made still less probable by the fact that all of the steps occur simultaneously for each peptide derivative. The only possible conclusion, therefore, is that a proton is displaced from each peptide linkage of both the di- and tripeptide. The titration data also prove that glycylglycylglycine is a tetradentate ligand for the copper(II) ion. It is interesting to note that the compound IV actually isolated is only an intermediate in the neutralization reaction indicated in Fig. 2, and that it contains a peptide hydrogen which may be neutralized by further reaction with alkali-metal hydroxide.

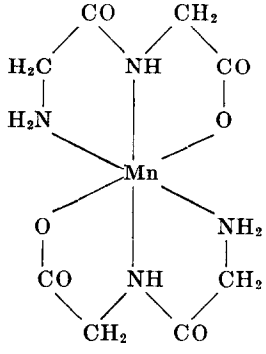
The analytical results obtained for the three cobalt(III) chelates prepared indicate formulas V, VI, and VII to be correct structures. The structure required by the constant coordination number 6, a nitrogen to cobalt ratio of 4:1, and the presence of an alkali-metal ion, can only be satisfied by formula V, in which a hydrogen ion is displaced from each peptide linkage. It is also interesting to note that, in accordance with formulas VI and VII, one mole of glycylglycine occupies four coordinate positions about the cobalt atom with the displacement of three hydrogen ions, two of which must come from the peptide linkages. In conformity with this arrangement of donor groups, the two remaining positions about the cobalt atom may be occupied by a bidentate glycinate anion, giving a complex with a single negative charge, or may accept two ammonia molecules, to produce a complex with a net charge of zero.

Formula VII indicates that nickel, with a coordination number of six, combines with two moles of glycylglycine and displaces two hydrogen ions from each. Here again, therefore, the peptide linkage acts as an acidic group in coordination with a metal ion. The nickel curve of Fig. 1 indicates that both amino and amide protons are displaced when two moles of glycylglycine combine with one mole of nickel ion. In this respect the behavior of nickel resembles somewhat the behavior of copper. Otherwise, however, the copper(II) and nickel(II) chelates differ considerably. The difference in stoichiometry is a consequence of the difference in coordination numbers of the two metal ions. Also, the titration curves of Fig. 1 indicate that the copper(II) chelate is much more stable than the nickel(II) derivative. Although copper chelates are generally more stable than the corresponding nickel chelates, the difference observed here seems to be much greater than is generally found with other ligands.

Metal chelates formed between glycylglycine and glycylglycylglycine and such metal ions as Mg(II) and Mn(II) have much lower stability than the copper(II) and nickel(II) analogs. In such compounds there is no dissociation of hydrogen ions from the peptide linkages. The titration curves obtained in the presence of the metal ion are only slightly below the curve of the ligand itself, and have inflection regions at one mole of base per mole of ligand. There is some evidence to indicate that the peptide linkage is involved as a donor group even for these relatively unstable compounds, in that the stabilities of the compounds indicated by formulas IX and X are significantly greater than the associations which occur with simple carboxylate ions or amino groups. Since no increase in stability would result from the coordination of both ends of the dipeptide to form an 8-membered ring, the peptide linkage must be involved in coordination with the metal ion.



IX



X

An attempt to prepare a 1:1 nickel(II) chelate of glycylglycylglycine by the methods outlined in the experimental part resulted in incomplete solution of the nickel(II) hydroxide and the formation of a compound having a nitrogen to metal ratio of 4.5:1. Although this substance requires more study before its structure can be discussed, it is perhaps significant that here the number of moles of ligand exactly matches the coordination requirements of the metal ion.

It should be noted that there is nothing in the evidence presented in this paper which indicates that other ratios of ligand to donor in the metal chelate compounds are not possible when the relative amounts of reacting species are changed. A difference in the formulation of such compounds, however, cannot affect the conclusions which have been drawn

above concerning the nature of the chemical binding between metal ion and ligand.

It is perhaps of interest to consider what bearing the results of this investigation can have on the binding of metal ions to proteins. First it is now apparent that the binding of copper(II) ions to polypeptides and proteins is very strong and nonspecific, since it conceivably would combine as effectively with a simple polyglycine as with a natural peptide. The binding of nickel(II) ions, and also possibly cobalt(II) and zinc(II) ions, would not be as strong as that of the copper(II) ion, but would be of the same general type. The more basic metals, however, such as Cd(II), Mn(II), and the alkaline-earth ions require more than simple peptide linkages for effective binding, and any stable combinations of such ions with a protein must occur through the agency of sterically favorable auxiliary groups which have appreciable affinity for the metal ion.

Summary

The preparation of copper(II), cobalt(III), and nickel(II) chelates of glyclyglycine and of glycylglycylglycine is described and the structures of the compounds are deduced with the aid of analytical data and titration curves. For both ligands it was found that association with these metal ions was accompanied by the displacement of a hydrogen ion from each peptide linkage.

References

1. Davies, C. W., and Waind, G. M., *J. Chem. Soc.* **1950,** 301.
2. Klotz, I. M., Faller, I. L., and Urquhart, J. M., *J. Phys. & Colloid Chem.* **54,** 18 (1950).
3. Monk, C. B., *Trans. Faraday Soc.* **47,** 285, 292, 297 (1951).
4. Monk, C. B., *Trans. Faraday Soc.* **47,** 1233 (1951).
5. Kober, P. A., and Sugiura, K., *Am. Chem. J.* **48,** 383 (1912).
6. Pfeiffer, P., and Saure, S., *J. prakt. Chem.* [2] **157,** 97 (1941).
7. Fischer, E., *Ber.* **34,** 433 (1901).
8. Fischer, E., "Untersuchungen uber Aminosauren, Poly peptides und Protein," Vol. I, p. 281. (1899–1906).